Role of aminoacyl-tRNA synthetases and viral RNA structure in the initiation of reverse transcription

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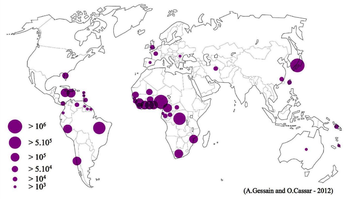
Background

- HTLV-1 is a deltaretrovirus that is the known cause of adult T-cell leukemia/lymphoma (ATL) and HTLV-1-associated myelopethy/tropical spastic paraparesis (HAM/TSP).
- Between 5-20 million people carry HTLV-1.
- Combining antiretrovirals and chemotherapy has had minor success, but there is still no cure for these diseases.
- **Hypothesis:** EPRS may be involved in HTLV-1 reverse transcription primer localization.
- These results will help to better understand a key viral regulatory mechanism, and may contribute to the future design of therapeutics.

Thé et al., AIDS Research and Human Retroviruses (1993)
Gessain et al., Frontiers in Microbiology (2012)

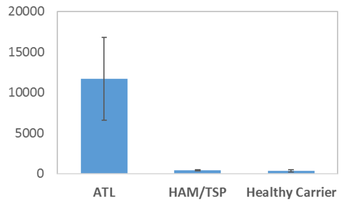
Proviral load reduction could lower ATL rates in endemic areas

World Distribution of HTLV-1 Infection



- HTLV-1 is distributed globally and endemic in areas such as West Africa, Japan, the Middle East, the Caribbean, and Brazil.
- High proviral load is a significant indicator of HTLV-1 progression to ATL.
- This implies that targeting drugs toward the virus could be successful in preventing the progression of viral infection to disease.

Proviral Load of HTLV-1 Diseases



Figures modified from Akbarin et al., Iranian Journal of Basic Medical Science (2013)

Retroviral life cycle

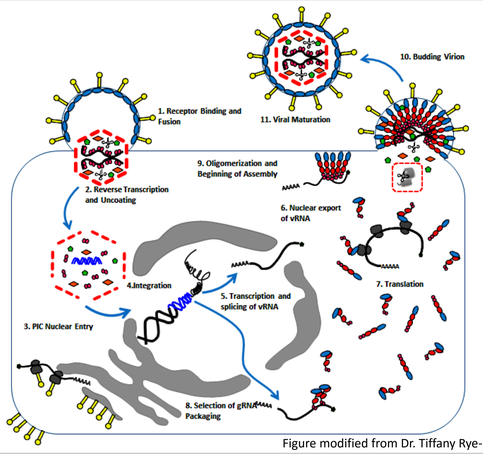
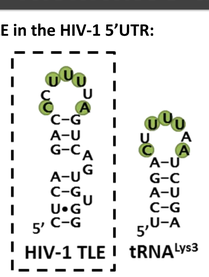
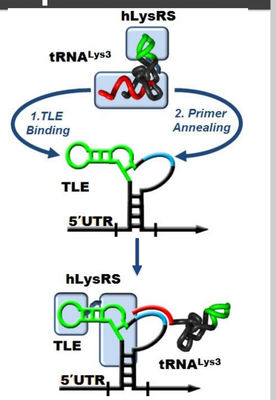


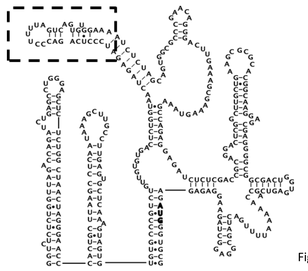
Figure modified from Dr. Tiffany Rye-McCurdy

TLE in HIV-1 binds LysRS and facilitates tRNA placement

TLE in the HIV-1 5'UTR:







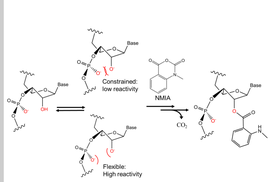
RNA	LysRS K_d (nM)
$tRNA^{Lys3}$	407 ± 33
PBS-TLE	383 ± 18
PBS + Psi-SL1	281 ± 25
PBS + Psi-SL1-SL2	85.4 ± 5.4

Figure Modified from Jones et al. RNA (2013)

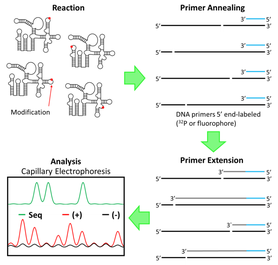
- HIV-1 utilizes a tRNA-like element (TLE) to localize $tRNA^{Lys3}$, the primer for reverse transcription, to the primer binding site (PBS).
- This mechanism and the preliminary data led us to hypothesize that the human bifunctional synthetase, EPRS, could be involved in HTLV-1 $tRNA^{Pro}$ primer localization.

New RNA probing data analysis method improves SHAPE

SHAPE Reaction:



Experimental Schematic:

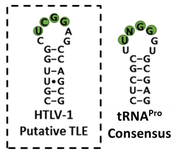


New Analysis Method:

Step 1: Signal Alignment	Size Standard Alignment	→	Analysis Window Selection	
Step 2: Preprocessing	Smoothing	→	Baseline Correction	→ Signal Decay Correction
Step 3: Sequence Alignment	Peak Picking	→	Sequence Alignment	
Step 4: Reactivity Calculation	Peak Area Approximation	→	Scaling	→ Normalization

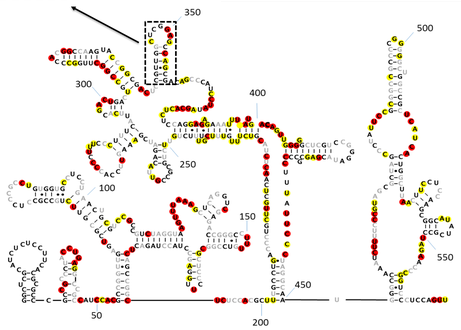
- Selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) uses reactive anhydrides to detect RNA flexibility at single-nucleotide resolution.
- Modifications were made to the analysis of capillary electrophoresis RNA probing data, primarily improving the signal alignment and peak picking steps.
- This improves the speed and accuracy of SHAPE data analysis.

SHAPE-solved HTLV-1 2° structure reveals potential TLE



Sequence Conservation of the Putative TLE

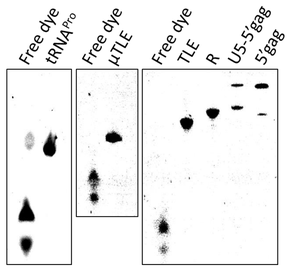
Sequence Type	Quantity	Percent
Identical Sequence	245	76.6
Stabilizing	53	16.6
Innocuous	5	1.6
Interfering	17	5.3
Total	320	



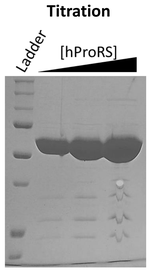
- Possible TLE identified in 2° structure: resembles the anticodon hairpin of $tRNA^{Pro}$, the primer for HTLV-1 reverse transcription.
- Putative TLE is highly conserved.

PAGE shows homogeneity of RNA and protein constructs

Urea PAGE: FTSC-Labeled RNA

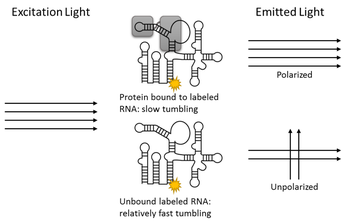


SDS PAGE: Protein Titration



Experimental schematics

Fluorescence anisotropy direct binding assay:



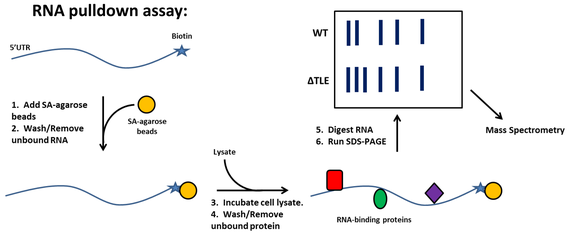
Anisotropy:

$$r = \frac{I_{||} - I_{\perp}}{I_{||} + 2I_{\perp}}$$

Hill Equation:

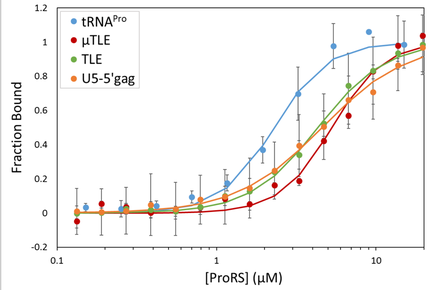
$$r([P]) = r_{min} + \frac{(r_{max} - r_{min})[P]^n}{K_d{}^n + [P]^n}$$

RNA pulldown assay:



RNA pulldown assay figure modified from Dr. William Cantara

Direct binding indicates that ProRS does not selectively bind HTLV-1 TLE



Binding Results:

RNA	ProRS K_d (µM)
$tRNA^{Pro}$	2.30 ± 0.20
µTLE	5.29 ± 0.36
TLE	4.32 ± 0.29
U5-5'gag	4.59 ± 0.62

- ProRS binds to the TLE containing constructs with nearly a two-fold greater K_d than $tRNA^{Pro}$.
- This weak binding constant suggests that the putative TLE would not compete well with $tRNA^{Pro}$ for ProRS binding, making this a poor tRNA-localizing element.

RNA pulldown detects new proteins

Top 5 Proteins Identified by MS

Protein	MW	Flu	5'UTR	ΔTLE
ACACA	266	132	172	64
NUCL	77	177	144	28
PTBP1	57	20	97	0
U2AF2	54	4	53	0
K2C1	66	168	46	101

- Mass spectrometry (MS) of proteins that were pulled down reveals novel interacting partners with the HTLV-1 5'UTR.
- Interestingly, polypyrimidine track binding protein 1 (PTBP1) and U2 small nuclear RNA auxiliary Factor 1 (U2AF1), were not detected when the putative TLE was deleted.
- However, MS did not reveal EPRS among the pulled-down proteins.

Conclusions and Future Directions

RNA probing analysis method and the HTLV-1 5'UTR

- Improvements in probing analysis led to the calculation of the secondary structure of the HTLV-1 5'UTR, revealing a potential tRNA-like element.

Experimental results

- Human ProRS domain binds two-fold tighter to $tRNA^{Pro}$ than to HTLV-1 RNAs containing the putative TLE.
- This suggests that this hairpin would not compete well with the tRNA for binding, a necessary function for primer localization.
- However, RNA pulldown also shows that other cellular proteins may be interacting with this region of the 5'UTR.

Future directions

- Explore *in vivo* experimentation with bifunctional EPRS.
- Investigate the proteins identified in RNA pulldown.